

EXHIBIT K

Levels of Household Mold Associated with Respiratory Symptoms in the First Year of Life in a Cohort at Risk for Asthma

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We assessed prospectively the risk of increased incidence of respiratory symptoms after exposure to particular fungal genera in a susceptible population—namely, infants ($n = 880$) at high risk for developing asthma. Days of wheeze or persistent cough, information on maternal allergy and asthma, socioeconomic variables, and housing characteristics were collected over the course of the infant's first year of life. Exposure to mold was assessed by airborne samples collected at one time early in the infant's life. Fungi were identified to genus level, recorded as colony-forming units per cubic meter (CFU/m³), and then categorized into four levels: 0 (undetectable), 1–499 CFU/m³ (low), 500–999 CFU/m³ (medium), and $\geq 1,000$ CFU/m³ (high). Effects of mold on wheeze and persistent cough, adjusting for potential confounding factors, were examined with Poisson regression analyses. The two most commonly found genera were *Cladosporium* (in 62% of the homes) and *Penicillium* (41%). *Cladosporium* was associated with reported mold ($p < 0.02$) and water leaks ($p < 0.003$). Rate of persistent cough was associated with reported mold [Rate ratio (RR) = 1.49; 95% CI, 1.18–1.88]. The highest level of *Penicillium* was associated with higher rates of wheeze (RR = 2.15; 95% CI, 1.34–3.46) and persistent cough (RR = 2.06; 95% CI, 1.31–3.24) in models controlling for maternal history of asthma and allergy, socioeconomic status, season of mold sample, and certain housing characteristics. We conclude that infants in this high-risk group who are exposed to high levels of *Penicillium* are at significant risk for wheeze and persistent cough. **Key words:** asthma, fungi, indoor air, infants, mold, *Penicillium*, wheezing. *Environ Health Perspect* 110:A781–A786 (2002). [Online 12 November 2002]

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Fungi are ubiquitous on this planet, and exposure to them a fact of life for all residents of Earth. There is little doubt that exposure to fungi contributes to respiratory diseases and their symptoms (Al-Doory and Domson 1984; Dillon et al. 1999; Husman 1996; Larone 1987; Peat et al. 1998; van der Werff 1958; Verhoeff and Burge 1997). The effect of indoor fungal exposures on the respiratory health of children is of particular concern and has been examined worldwide. Studies have been primarily of children recruited directly from schools in Canada (Dales et al. 1999), the Netherlands (Verhoeff et al. 1995), Sweden (Rylander et al. 1998), and Taiwan (Su et al. 2001b), or children who were members of surveyed families in Australia (Garrett et al. 1998), Israel (Katz et al. 1999), and the United Kingdom (Platt et al. 1989). A few studies have specifically examined the effects of fungal exposure on children with respiratory or allergic disease (Dill and Niggemann 1996; Li et al. 1995; Su et al. 2001a).

Several methods are used to assess levels of mold exposure, including counting fungal spores in airborne samples or quantifying constituents of fungal cells contained in dust samples. Compared to dust sample analyses that can quantify cumulative exposure to mold in general (e.g., Chew et al. 2001; Dharmage et al. 2001; Douwes et al. 1999), air sampling

remains the best way to identify genera and species of molds found in and around a home (Ren et al. 1999). Relationships between exposure to mold and respiratory symptoms of children have been explored for airborne samples analyzed as total mold spore counts (Platt et al. 1989; Strachan et al. 1990; Waegemaekers et al. 1989), and as concentrations of specific genera (e.g., *Cladosporium*, *Penicillium*, *Alternaria*, *Aspergillus*) (Garrett et al. 1998; Li et al. 1995; Su et al. 2001a).

No studies have reported on mold exposures in early life and respiratory symptoms in a susceptible population. Since 1998, we have been following a birth cohort of 1,002 infants at risk for developing asthma. We have reported previously on the relationship between airborne fungi and characteristics of our study subjects' homes (Ren et al. 2001). In the present study we examine which specific mold exposures might be related to respiratory symptoms while controlling for potentially confounding factors.

Methods

Cohort. We studied 1,002 infants born to mothers in Connecticut and western Massachusetts between September 1996 and December 1998, who had at least one older child with physician-diagnosed asthma. The Human Investigation Committee of Yale

University approved this study, and all respondents gave informed consent before participation.

Because they all had at least one sibling with asthma, the infants in this birth cohort were considered to be at high risk for developing asthma. Within 4 months of the infant's birth, a trained research assistant interviewed each mother at home to collect demographic information, medical histories, and household characteristics. Mothers were asked whether the infant had experienced wheeze or persistent cough since birth and, if so, how many days in each month the symptoms occurred. Infant respiratory symptoms were collected at quarterly telephone interviews when the infant was 6, 9, and 12 months of age. When the infant was 12 months old, a more extensive questionnaire was administered covering additional household characteristics specific to the previous year, including the respondent's observation of persistent mold or mildew in the living area of the home.

Of the initial 1,002 infants enrolled, 880 were included; for these, symptom information was available for 1 to 12 months in the home from which the mold sample was taken. Excluded were 39 families who moved at some unknown date from the home where the mold sample was taken, 54 who were lost to follow-up, and an additional 29 who were missing information on measured mold. Data from these 880 infants were used in analyses of general characteristics of the infant and home collected at the time of the home interview. Data from a subset of 819 infants whose mothers participated in the year 1 questionnaire were used for analyses including housing characteristics specific to the infant's first year of life.

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Collection and analysis of mold samples.

Sample collection and analysis have been described previously (Ren et al. 2001). Results reported here are from airborne mold samples collected from the main living area at the time of the home visit within 4 months of the infant's birth. A Burkard portable air sampler (Burkard Manufacturing Co., Rickmansworth, UK) was used in combination with dichloran-18% glycerol agar (DG-18). The sampling period was 1 min with an airflow rate of 20 L/min. Fungi were identified to the genus level and recorded as colony-forming units (CFU) per cubic meter.

Data analysis. The health outcomes of interest were infant respiratory symptoms of wheeze and persistent cough measured as counts (i.e., number of days of symptom summed over the period of time that the infant lived in the home where the mold sample was taken). Symptom counts were converted to yearly rates by dividing number of days of symptoms by the number of days of observation and multiplying times 365 days per year. For some analyses, yearly rates were categorized into three levels previously established for an asthma severity scale (Ortega et al. 2001): 0 days, < 30 days/year, \geq 30 days/year.

The total culturable number of colony-forming units per cubic meter was categorized into four levels according to the Commission of European Communities report (CEC 1994): 0 (undetectable), 1–499 CFU/m³ (low), 500–999 CFU/m³ (medium), and \geq 1,000 CFU/m³ (high). Exposure variables of interest for further analyses included all specifically identified genera of fungi. Total amount of yeast, which is not speciated, was noted but not included in further analyses.

Other variables of interest were collected at the home interview ($n = 880$), including personal and socioeconomic information (infant sex, ethnicity, maternal history of asthma and allergies, mother's education level, multifamily home), other housing characteristics (smoking in the home, heating system), and season mold sample was taken: winter (the months of December–February), spring (March–May), summer (June–August), and fall (September–November). Additional information on housing characteristics specific to the infant's first year of life in the home where airborne mold was sampled were collected at year 1 interviews ($n = 819$) and included reports of mold and mildew, problems with water leaks, and use of humidifiers and/or dehumidifiers.

Because wheeze and persistent cough data are in the form of counts (number of days of symptoms), we used Poisson regression analysis to examine associations between symptoms and mold variables, both unadjusted and adjusted for potential confounding factors. Rate ratios (RR) and 95% confidence intervals (CI) were computed using the GENMOD procedure in

SAS specifying the Poisson distribution and invoking the scaling factor correction for overdispersion (SAS 2001). Because not all infants were under observation for a full year, and to standardize the length of the monitoring period, we added an offset variable (log of the number of days during the first year of life that the infant was under observation) to the model (Allison 1999; SAS 2001). Models were built for the simultaneous effects of *Penicillium*, *Cladosporium*, and "other" mold, using backward elimination of nonsignificant confounders, with reinclusion if the confounder's exclusion produced > 10% change in the coefficients of the exposure variables. For each mold variable, each category of mold exposure (low, intermediate, high) was entered into the models as a dummy variable with the "undetectable" category serving as baseline.

Results

Overall, 80% of the infants remained in the study home for 1 full year, 12% were followed for 9–11 months, 5% for 6–8 months, and

3% for fewer than 6 months. Over half of the infants did not experience any wheeze (58.8%) or persistent cough (54.3%). Approximately one-third of the infants who experienced symptoms had the equivalent of 30 or more days of wheeze (27.5% of 402 infants) or persistent cough (32.8% of 363).

Table 1 displays the unadjusted relationships between personal, socioeconomic, and housing characteristics and the rates of wheeze and persistent cough. There is significantly more wheeze and persistent cough among boys (χ^2 , $p < 0.04$) and among infants whose mothers have asthma ($p < 0.002$). Higher rates of wheeze are found among Hispanic infants ($p < 0.007$), infants of mothers with less than a high school education ($p < 0.004$), and infants living in multifamily housing ($p < 0.001$). Infants in homes heated with an electric baseboard system tend to have higher rates of wheeze than those in homes with forced air, steam, or any other system ($p < 0.01$). Rates of respiratory symptoms were not associated with smoking

Table 1. Unadjusted associations between personal, socioeconomic, housing characteristics, and respiratory symptoms of 880 infants at risk for developing asthma from Connecticut and western Massachusetts, 1998–2000.

Characteristic	No.	Rate of persistent cough (days/year) (%)				Rate of wheeze (days/year) (%)			
		0	< 30	\geq 30	p -Value ^a	0	< 30	\geq 30	p -Value ^a
Infant's sex									
Male	437	50.1	34.1	15.8	0.04	54.5	31.8	13.7	0.02
Female	443	58.5	27.3	14.2		63.0	28.0	9.0	
Ethnicity									
White, Asian, other	554	54.7	31.8	13.5	0.08	61.4	30.1	8.5	0.007
Black	120	60.0	28.3	11.7		57.5	29.2	13.3	
Hispanic	206	50.0	29.1	20.9		52.4	29.6	18.0	
Mother has diagnosed asthma									
No	601	58.6	29.1	12.3	0.001	62.6	28.0	9.5	0.002
Yes	279	45.2	34.0	20.8		50.5	34.0	15.4	
Mother has allergies									
No	371	57.4	28.3	14.3	0.28	61.2	29.9	8.9	0.13
Yes	509	52.1	32.4	15.5		57.0	29.9	13.2	
Mother's education (years)									
< 12	125	54.4	24.6	20.0	0.34	50.4	28.8	20.8	0.004
12–15	463	53.8	31.1	15.1		59.4	29.4	11.2	
> 15	292	55.1	32.9	12.7		61.3	31.2	7.5	
Multifamily home									
No	716	54.6	31.3	14.1	0.28	60.5	30.0	9.5	0.001
Yes	164	53.0	28.0	18.9		51.2	29.3	19.5	
Smoking in the home									
No	761	53.2	31.1	15.6	0.20	59.3	29.7	11.0	0.65
Yes	119	61.3	27.7	10.9		55.5	31.1	13.4	
Heating system									
Forced air	309	58.2	25.6	16.2	0.18	61.5	28.8	9.7	0.01
Steam/hot water	419	51.3	33.9	14.8		59.0	30.8	10.3	
Electric	123	55.1	30.9	13.0		53.7	26.0	20.3	
Other	24	41.7	45.8	12.5		45.8	50.0	4.2	
Water leaks ^b									
No	636	56.0	29.7	14.3	0.10	60.4	30.0	9.6	0.45
Yes	183	47.0	36.6	16.4		55.2	33.9	10.9	
Humidifier use ^b									
No	377	56.8	31.0	12.2	0.12	60.2	31.8	8.0	0.23
Yes	442	51.6	31.4	17.0		58.4	30.1	11.5	
Dehumidifier use ^b									
No	597	52.3	31.7	16.1	0.15	57.3	31.7	11.1	0.09
Yes	222	58.6	30.2	11.3		64.4	28.8	6.8	

^a p -Values for χ^2 tests. ^bData from questionnaire administered at the infant's first birthday ($n = 819$).

in the home, mother's history of allergies, reported water leaks in the home, or the use of a humidifier or dehumidifier.

Genera of mold identified in the main living room of 880 homes included *Cladosporium* (found in 61.9% of the homes) and *Penicillium* (in 40.6%). Although *Aspergillus* (in 25.6%) and *Alternaria* (12.1%) were also quite common, neither was found in sufficient numbers to be analyzed separately. An additional 21 genera were identified including *Wallemia* (7.5%), *Epicoecum* (5.9%), *Botrytis* (3.1%), and others found in less than 3% of the homes. No mold could be detected in the main living room of 15.6% of the homes. The number of different genera per sample in the remaining samples ranged from 1 to 7 (median = 2 different molds). *Cladosporium* was the only mold identified in 11.7% of the samples while *Penicillium* was the only mold in 7.8%. Both were present in 27.3% of the main room samples. Unspecified yeast was found in 44.2% of the samples, and it was not possible to identify at least one of the molds present on 24.4% of the samples.

Table 2 displays bivariate associations between season of mold sampling, housing characteristics, and measured mold. There is no association between season of sampling and level of *Penicillium*. There is a significant association between season and level of *Cladosporium* and "other" mold ($p < 0.0001$). Only *Cladosporium* is associated with the mother's observation of mold ($p < 0.02$) or water leaks ($p < 0.003$) during the first year of the infant's life. Higher levels of "other" mold were associated with steam/hot water home heating systems ($p < 0.05$).

For the infants who experienced respiratory symptoms, Figure 1 shows the frequency distributions for days of wheeze and persistent cough in the first year of life (expressed as the rate multiplied by 365 days) over each level of *Penicillium* and *Cladosporium*. An examination of the tail of the distribution for days of wheeze in the presence of *Penicillium* reveals that although only 19 of 363 infants were exposed to high levels of this mold, 37% of them experienced 60 or more days of wheeze in their first year of life (Figure 1B). Interestingly, only 3% of 29 infants exposed to high levels of *Cladosporium* had this same high rate of wheeze (Figure 1D). The distribution for days of persistent cough shows a similar pattern in the tails: More infants experience 60 or more days of persistent cough in the presence of high levels of *Penicillium* (5 of 18, or 28%) (Figure 1A) than do in the presence of high levels of *Cladosporium* (0 of 31) (Figure 1C).

Table 3 shows the unadjusted associations resulting from a Poisson regression analysis between the rates of respiratory symptoms and exposure to particular levels of molds. As illustrated in Figure 1, high levels of *Penicillium* are significantly associated with wheeze (RR = 2.46; 95% CI, 1.63–3.70) and persistent cough (RR = 1.84; 95% CI, 1.22–2.80). In addition, persistent cough is also significantly associated with the medium level of *Cladosporium* (RR = 1.62; 95% CI, 1.17–2.24) and with mother's report of mold in the home (RR = 1.49; 95% CI, 1.18–1.88).

The simultaneous effects of *Penicillium*, *Cladosporium*, and "other" mold adjusted for potential confounding factors are displayed in the Poisson regression models in Table 4. The

presence of *Penicillium* appears to increase the chances that an infant will experience significant respiratory symptoms during the first year of life. The model for rate of persistent cough suggests that when controlling for maternal history of asthma, mother's education level, season of mold sample, water leaks, humidifier or dehumidifier use, and smoking in the home, an infant's number of days of persistent cough during the first year of life in the presence of a high level of *Penicillium* would be double the number experienced in the presence of undetectable levels of this mold (RR 2.06; 95% CI, 1.31–3.24). It appears that when controlling for confounding factors and level of *Penicillium*, neither *Cladosporium* nor "other" mold affects respiratory symptoms experienced by an infant during the first year of life.

Similarly, the model for rate of wheeze suggests that when controlling for the factors mentioned above plus the infant's sex, maternal history of allergies, multifamily home, and home heating system, an infant's number of days of wheeze in the first year of life in the presence of high levels of *Penicillium* would again be double the number experienced when unexposed to *Penicillium* (RR = 2.15; 95% CI, 1.34–3.46).

Ethnicity and use of air conditioners were included in initial models predicting rates of wheeze and persistent cough, but were eliminated because they had no confounding effects on the remaining variables (i.e., > 10% change in the parameter estimate of interest).

Discussion

We assessed the risk of increased incidence of respiratory symptoms as a result of exposure to particular fungi in a susceptible population—

Table 2. Unadjusted associations between season of sampling, housing characteristics, and mold measured in 880 homes 2–4 months after an infant's birth (Connecticut and western Massachusetts, 1998–2000).

Variable	No.	<i>Penicillium</i> ^a (%)					<i>Cladosporium</i> ^a (%)					Other mold ^a (%)				
		0	Low	Med	High	p-Value ^b	0	Low	Med	High	p-Value ^b	0	Low	Med	High	p-Value ^b
Season mold sample taken																
Winter	181	66.8	26.5	1.7	5.0	0.51	71.3	27.1	1.7	0.0	0.0001	61.3	33.7	1.1	3.9	0.0001
Spring	244	59.8	33.6	2.5	4.1		57.0	37.7	2.9	2.5		49.6	48.4	1.6	0.4	
Summer	219	56.6	37.4	2.7	3.2		12.3	48.2	21.9	17.4		26.5	63.0	7.3	3.2	
Fall	236	55.9	37.3	2.5	4.2		17.0	63.1	12.7	7.2		32.3	57.2	7.2	3.4	
Heating system																
Forced air	309	61.8	32.0	2.9	3.2	0.85	41.0	44.0	8.7	6.2	0.43	46.6	45.6	6.2	1.6	0.05
Steam/hot water	419	57.0	35.8	2.6	4.5		36.8	44.2	11.9	7.2		40.8	52.0	3.3	3.8	
Electric	123	61.0	33.3	0.8	4.9		33.3	50.4	8.1	8.1		32.5	61.8	4.1	1.6	
Other	24	58.3	37.5	0.0	4.2		50.0	41.7	0.0	8.3		37.5	58.3	4.2	0.0	
Reported mold ^c																
No	644	60.9	33.5	2.2	3.4	0.30	38.8	46.3	9.3	5.6	0.02	42.7	50.8	4.0	2.5	0.42
Yes	174	54.0	37.4	3.4	5.2		35.1	40.8	12.6	11.5		36.8	54.0	5.8	3.4	
Water leaks ^c																
No	636	59.3	34.1	2.7	3.9	0.83	37.9	46.9	8.0	7.2	0.003	42.3	50.9	4.4	2.4	0.64
Yes	183	59.6	35.5	1.6	3.3		38.2	39.3	16.9	5.5		38.8	53.0	4.4	3.8	
Humidifier use ^c																
No	377	57.8	34.5	2.4	5.3	0.21	38.5	45.1	10.6	5.8	0.72	41.9	51.5	3.4	3.2	0.55
Yes	442	60.6	34.4	2.5	2.5		37.6	45.2	9.5	7.7		41.2	51.4	5.2	2.3	
Dehumidifier use ^c																
No	597	59.1	35.0	2.8	3.0	0.16	37.4	46.4	10.6	5.7	0.12	41.2	52.4	4.0	2.4	0.54
Yes	222	59.9	32.9	1.4	5.9		39.6	41.9	8.6	9.9		42.3	48.6	5.4	3.6	

^aUndetectable: 0; low: 1–499 CFU/m³; medium: 500–999 CFU/m³; high: ≥ 1,000 CFU/m³. ^bp-Values for χ^2 tests. ^cData from questionnaire administered at the infant's first birthday ($n = 819$).

namely, infants at high risk for developing asthma. Our assessment also considers factors that might have confounding effects, such as maternal allergy and asthma, socioeconomic variables, and housing characteristics. We conclude that infants in this high-risk group who are exposed to higher levels of *Penicillium* are at significant risk for wheeze and persistent cough.

Previous studies suggest that the relationship between exposure to mold and respiratory symptoms of children is complicated and may depend on risk level of the study population, health outcomes measured, exposure assessment methodology, and other factors identified as potentially confounding. Results of previous studies examining respiratory symptoms of children and indoor exposure to measured airborne mold as represented by total mold spore count are equivocal. Platt et al. (1989) used airborne mold sampling to confirm observed mold and found that children living in homes classified as damp and moldy had significantly more respiratory symptoms than did children in dry homes. In a study of 88 7-year-olds from a population survey of 1,000 homes in the United Kingdom, Strachan et al. (1990) used measured mold as the exposure variable and concluded that inhaled mold spores (total mold count) is not an important factor in causing wheeze. On the other hand, Waegemaekers et al. (1989) studied 190 school-aged children in the Netherlands and

for a subset of 56 children reported an association between the presence of exposure to mold (modeled as the \log_{10} of total mold count in CFU/m³) and persistent cough [odds ratio (OR) = 1.98; $p < 0.05$] and a combination of wheeze and persistent cough (OR = 1.93; $p < 0.05$), adjusted for socioeconomic variables and household characteristics.

Results of studies examining the relationship between exposure to specific mold genera and respiratory symptoms have also been equivocal. Garrett et al. (1998) took repeated airborne samples from 80 homes of 148 7–14-year-olds and concluded that an increase in winter exposure to *Penicillium* by 100 CFU/m³ (OR = 1.43; 95% CI, 1.03–2.00),

Table 3. Unadjusted associations estimated as RRs and 95% CIs between measured mold, reported mold, and respiratory symptoms of 880 infants (Connecticut and western Massachusetts, 1998–2000).

Factor	No.	Persistent cough RR (95% CI)	Wheeze RR (95% CI)
Measured mold ^a			
<i>Penicillium</i>			
Undetectable (R)	52		
Low	300	1.07 (0.86–1.34)	1.06 (0.82–1.36)
Medium	21	1.67 (0.96–2.93)	1.10 (0.51–2.34)
High	36	1.84 (1.22–2.80)*	2.46 (1.63–3.70)*
<i>Cladosporium</i>			
Undetectable (R)	335		
Low	396	1.17 (0.92–1.47)	1.12 (0.87–1.45)
Medium	88	1.62 (1.17–2.24)*	1.07 (0.71–1.61)
High	61	0.75 (0.45–1.24)	0.83 (0.50–1.40)
"Other" mold ^b			
Undetectable (R)	339		
Low	421	1.24 (1.00–1.54)	1.31 (1.00–1.63)
Medium	36	0.78 (0.42–1.45)	1.13 (0.63–2.03)
High	22	1.14 (0.60–2.16)	0.88 (0.39–1.98)
Reported mold ^c			
No (R)	644		
Yes	174	1.49 (1.18–1.88)*	1.23 (0.94–1.61)

R, reference category.

^aUndetectable: 0 CFU/m³; low: 1–499 CFU/m³; medium: 500–999 CFU/m³; high: $\geq 1,000$ CFU/m³. ^b"Other" mold defined as total spore counts minus counts for *Penicillium*, *Cladosporium*, and yeast. ^cData from questionnaire administered at the infant's first birthday ($n = 819$). * $p < 0.05$.

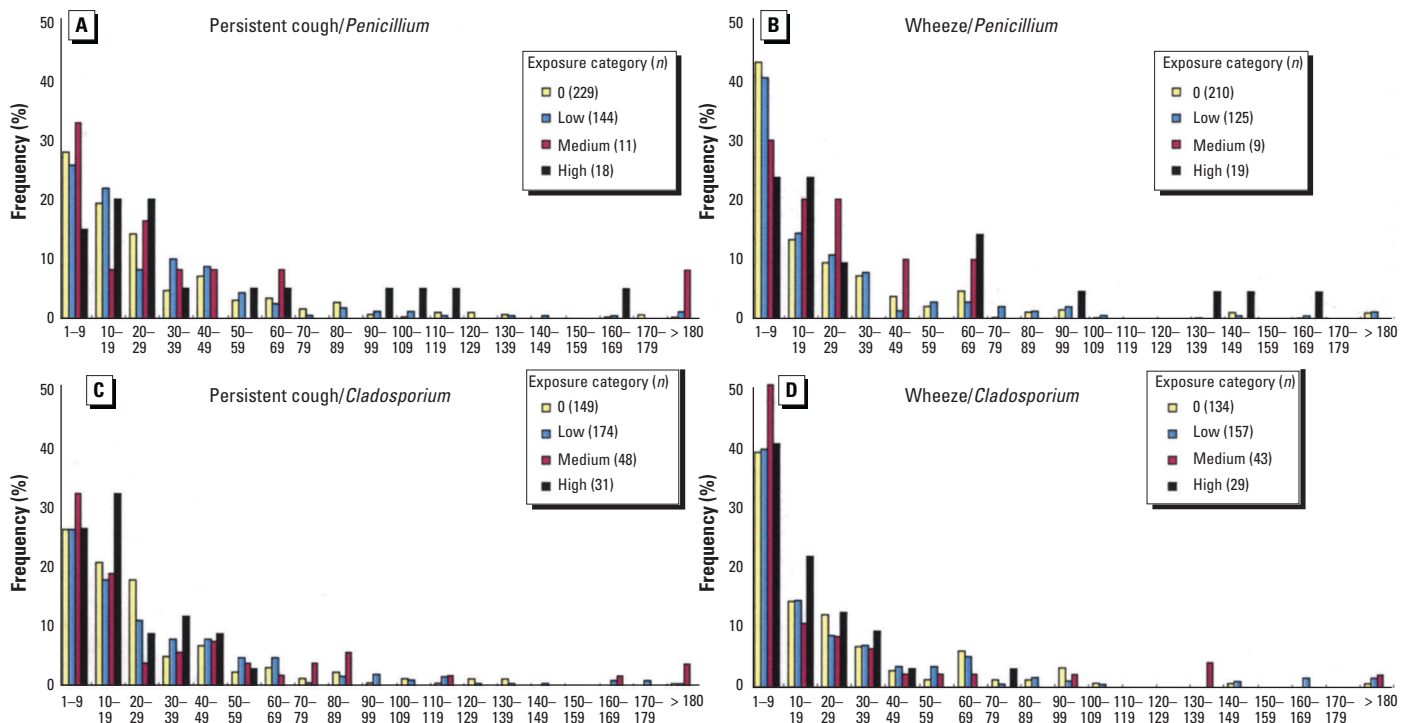


Figure 1. Rate of respiratory symptoms per year for infants who experienced any persistent cough (45.7%) (A,C) or wheeze (41.2% of 880) (B,D) in the first year of life. Because not all infants were followed for a full year, respiratory symptom rates, expressed as number of days of symptoms per days of follow-up, were multiplied by 365 days to produce a rate per year. Distribution of symptoms is shown for each exposure category of *Penicillium* (A,B) and *Cladosporium* (C,D). Data from Connecticut and western Massachusetts, 1998–2000.

adjusted for parental asthma and allergy, was related to asthma. Two studies compared the concentration of specific genera of airborne mold in the homes of children similar in age with and without asthma. One found significant differences in the concentration distributions of *Cladosporium* and *Penicillium* between disease and control groups (Li et al. 1995), and the other found no consistent pattern between groups for the various genera of fungi (Su et al. 2001a).

Interestingly, and consistent with our study findings that mold and water leaks are significantly associated with *Cladosporium* but not *Penicillium*, Garrett et al. (1998) also found that visible mold and evidence of condensation problems were associated with high concentrations of *Cladosporium*.

A major strength of our study is that we collected extensive respiratory symptom data,

maternal asthma and allergy histories, and housing characteristic information on a large population at high risk for developing asthma. Another strength is that we were able to examine associations between respiratory symptoms and exposure to two of the most common types of mold through the use of airborne mold sampling, which remains the best way to identify specific genera.

Limitations of our study come primarily from our mold sampling methodology. Our combination of air sampler and agar are considered to produce the best results in terms of precision, yield, and number of species isolated (Verhoeff et al. 1990). But, as with any air sampling method, spores from molds that are rare or that are not easily airborne will result in missed fungal identifications, as will spores that grow with difficulty or not at all in the chosen medium. Despite this, we feel

that our method accurately reflects the variety of molds present in the homes of our study subjects.

A more serious limitation is that a single airborne sample is used to represent mold exposure during the first year of life. Airborne mold samples, used in many studies as the measure of exposure, are quite variable over time [e.g., seasonal variation (Beaumont et al. 1984; Gravesen 1972; Ren et al. 1999)] and space [i.e., indoors vs. outdoors (Beaumont et al. 1984) or between locations indoors, e.g., basements vs. living rooms (Ren et al. 1999)] and as such have drawn criticism when used to represent longer-term levels of exposure (Verhoeff and Burge 1997). Estimates of cumulative exposure to mold from a single point-in-time sample are provided by dust sample analyses of ergosterol (Dharmage et al. 2001) or extracellular polysaccharide (Chew et al. 2001; Douwes et al. 1999). However, results of dust sample analyses are relatively nonspecific because they involve quantification of the aggregate fungal biomass of any molds.

All of the mold samples used in this report were taken from the main living room of the home, but were not all collected at the same time of year. Roughly one-fourth were taken in each season (21% winter, 25% summer, 27% fall, 28% spring), and there were no significant seasonal differences in the distribution of *Penicillium* levels (Ren et al. 1999; Table 3). Furthermore, a year-long study of repeated mold sampling in 11 homes in the same geographic area as our study (southern New England) demonstrated no significant variation in the concentration of total fungi in the living room between spring, summer, and fall, or between winter, spring, and fall, and very little seasonal variation in *Penicillium* as a percentage of total mold concentration (Ren et al. 1999). Thus, the concentration of *Penicillium* taken at one time is likely to be representative of *Penicillium* levels in the living room of a home in this area throughout the year. In contrast, the highest values of *Cladosporium* from the main living area in our study are from the summer samples (a time of year when *Cladosporium* reaches its peak levels outdoors) and lowest from the winter (Table 3); hence the importance of including season of sampling as a confounding factor in the models.

To the extent that our levels of measured mold sampled on one occasion represent longer-term exposure concentrations, it appears that risk of higher rates of respiratory symptoms increases with levels of *Penicillium* (Table 4). This linear trend proved to be significant when level of *Penicillium* was entered into the adjusted models as a quasi-continuous variable for both wheeze (RR = 1.23; 95% CI, 1.06–1.42) and persistent cough (RR = 1.21; 95% CI, 1.06–1.38). This result implies that

Table 4. Estimates of RRs and 95% CIs from Poisson regression models for measured mold related to wheeze and persistent cough in the first year of life adjusted for socioeconomic factors and housing characteristics^a (Connecticut and western Massachusetts, 1998–2000).

Factor	Model for persistent cough RR (95% CI)	Model for wheeze RR (95% CI)
Measured mold ^b		
<i>Penicillium</i>		
Undetectable (R)		
Low	1.01 (0.80–1.28)	1.11 (0.87–1.42)
Medium	1.62 (0.93–2.82)	1.29 (0.65–1.48)
High	2.06 (1.31–3.24)*	2.15 (1.34–3.46)*
<i>Cladosporium</i>		
Undetectable (R)		
Low	1.03 (0.79–1.35)	0.92 (0.69–1.22)
Medium	1.45 (0.99–2.12)	0.95 (0.61–1.49)
High	0.72 (0.42–1.24)	0.91 (0.53–1.56)
"Other" mold ^c		
Undetectable (R)		
Low	1.05 (0.83–1.33)	0.97 (0.75–1.26)
Medium	0.78 (0.42–1.45)	0.91 (0.49–1.68)
High	1.18 (0.63–2.21)	1.02 (0.49–2.11)
Season of mold sample		
Summer (R)		
Fall	0.85 (0.64–1.13)	1.00 (0.73–1.38)
Winter	0.75 (0.54–1.02)	0.87 (0.59–1.29)
Spring	0.74 (0.53–1.08)	0.81 (0.57–1.15)
Water leaks	1.17 (0.91–1.49)	1.18 (0.90–1.55)
Humidifier use	1.26 (1.01–1.56)*	1.41 (1.11–1.79)*
Dehumidifier use	0.80 (0.61–1.04)	0.83 (0.61–1.13)
Mother has asthma	1.49 (1.20–1.85)*	1.40 (1.10–1.78)*
Mother's education level (years)		
< 12	1.33 (0.92–1.91)	1.87 (1.25–2.80)*
12–15	1.06 (0.83–1.36)	1.20 (0.90–1.60)
> 15 (R)		
Smoking in the home	0.75 (0.53–1.08)	0.88 (0.62–1.25)
Heating system		
Forced air (R)		
Steam/hot water		0.89 (0.68–1.15)
Electric		1.30 (0.93–1.82)
Other		0.43 (0.15–1.19)
Male infant		1.60 (1.26–2.02)*
Mother has allergies		1.23 (0.97–1.58)
Multifamily home		1.50 (1.10–2.02)*

R, reference category.

^aOutcome data were entered into the model as the number of days of symptoms relative to the number of days the infant was under observation. Two additional variables (ethnicity and use of air conditioners) were included in initial models predicting rates of wheeze and persistent cough, but both were eliminated and neither had confounding effects on the remaining variables. ^bUndetectable: 0; low: 1–499 CFU/m³; medium: 500–999 CFU/m³; high: ≥ 1,000 CFU/m³. ^c"Other" mold defined as total spore count minus counts for *Penicillium*, *Cladosporium*, and yeast. **p* < 0.05.

an infant's number of days of respiratory symptoms during the first year of life would increase by 20% for each increase in the level of *Penicillium* (i.e., from no mold to a low level, or from low to intermediate, or from intermediate to high). It also appears that, when controlling for potential confounding factors including season the mold sample was taken, the association between respiratory symptoms and mold exposure holds true for *Penicillium* but not for *Cladosporium* or "other" mold. That there are significant seasonal variations in some molds suggests that exposure to *Cladosporium* is intermittent and may contribute only intermittently to respiratory symptoms and not as much as *Penicillium*, which appears to be present at more consistent levels year round.

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